Developmental Biology **237,** 1–17 (2001)
doi:10.1006/dbio.2001.0350, available online at http://www.idealibrary.com on IDEAL®

Early Posterior/Ventral Fate Specification in the Vertebrate Embryo

Ignacio Muñoz-Sanjuán and Ali H.-Brivanlou[1]
Laboratory of Vertebrate Embryology, The Rockefeller University, New York, New York 10021

One of the central questions in developmental biology is that of how one cell can give rise to all specialized cell types and organs in the organism. Within the embryo, all tissues are composed of cells derived from one or more of the three germ layers, the ectoderm, the mesoderm, and the endoderm. Understanding the molecular events that underlie both the specification and patterning of the germ layers has been a long-standing interest for developmental biologists. Recent years have seen a rapid advancement in the elucidation of the molecular players implicated in patterning the vertebrate embryo. In this review, we will focus solely on the ventral and posterior fate acquisition in the ventral–lateral domains of the pregastrula embryo. We will address the embryonic origins of various tissues and will present embryological and experimental evidence to illustrate how "classically defined" ventral and posterior structures develop in all three germ layers. We will discuss the status of our current knowledge by focusing on the African frog *Xenopus laevis*, although we will also gather evidence from other vertebrates, where available. In particular, genetic studies in the zebrafish and mouse have been very informative in addressing the requirement for individual genes in these processes. The amphibian system has enjoyed great interest since the early days of experimental embryology, and constitutes the best understood system in terms of early patterning signals and axis specification. We want to draw interest to the embryological origins of cells that will develop into what we have collectively termed "posterior" and "ventral" cells/tissues, and we will address the involvement of the major signaling pathways implicated in posterior/ventral fate specification. Particular emphasis is given as to how these signaling pathways are integrated during early development for the specification of posterior and ventral fates.

INTRODUCTION

A major issue in discussing the establishment of dorsal/ventral and anterior/posterior fate specification in vertebrates is that there is considerable debate about the full extent of the embryological origins of tissues labeled as "dorsal" or "ventral." It is important to keep in mind that traditionally termed "dorsal tissues" (such as the nervous system in the ectoderm or the somitic mesoderm) also have an anterior–posterior axis, and cells residing in different positions along that axis may have distinct embryonic origins. Similarly, it is difficult to reconcile the distinct embryonic origins of these cells with current molecular models that propose candidate molecules for the specification and patterning of a particular tissue in the embryo. That is, there is a need to correlate the embryonic origins of various cell types with the available molecular maps in the context of time and space at the critical periods in the specification of the various axis.

In *Xenopus*, the ectoderm arises from the animal (pigmented) pole and the endoderm from the vegetal pole of the embryo. The mesoderm arises in the marginal zone, which occupies an equatorial position, as a result of inductive interactions between the animal and vegetal poles of the embryo (Nieuwkoop, 1969a; Keller, 1991). Cells that become mesoderm and endoderm largely share a common origin, although to different extents in amphibians and other vertebrates, and this population of cells is termed mesendoderm (see Warga and Nusslein-Volahard, 1999; Grapin-Botton and Melton, 2000; Rodaway and Patient, 2001). The mechanisms by which these cells adopt one fate or the other are unclear (see Wells and Melton, 1999; and discussion below) but not all mesodermal cells derive from

[1] To whom correspondence should be addressed. Fax: (212) 327-8685. E-mail: brvnlou@rockvax.rockefeller.edu.

0012-1606/01 $35.00

the mesendoderm (see Rodaway and Patient, 2001). Within each layer, molecular signals and morphogenetic movements establish a set of coordinates along the anterior–posterior, dorsoventral, and left–right axis. By definition, the dorsal side of the amphibian embryo is the region where the blastopore lip forms. By contraposition, the ventral-most side is the opposite area in the embryo (see Fig. 1). In the ectoderm, cells located dorsally in the region overlaying the organizer will become neural tissue, whereas cells located in a more ventral position will become epidermis. In the mesoderm, cells located 60° radially form the organizer will become dorsal mesoderm. Dorsal marginal zone (DMZ) cells contribute to the entire dorsal midline of the embryo along the anterior–posterior axis, and their progeny give rise to axial (notochord), paraxial (muscle) mesoderm, heart, and head mesoderm. The rest of the 300° of the marginal zone is termed the ventral–lateral marginal zone (VLMZ). Lateral mesodermal derivatives are the lateral plate mesoderm, pronephros, some muscle, and blood (classically defined as ventral-most mesoderm, although see discussion below; Keller, 1975, 1976, 1991; Dale and Slack, 1987). As we will discuss below, the subdivision of the ventral–lateral region into distinct domains of gene expression has been poorly characterized, and it is assumed that ventral fates develop in the absence of signals emanating from the powerful dorsal organizer. Within the endoderm, cells located dorsally will become the pancreas. The gut and organs derived as outgrowths of the gut, the lungs, and liver, can be subdivided along its trajectory in the anterior–posterior axis (see Chambers and Slack, 2000; Wells and Melton, 1999; Grapin-Botton and Melton, 2000). Much emphasis has been placed in elucidating the signals that regulate dorsal and anterior development, whereas the formation of posterior and ventral fates needs closer scrutiny.

Fate Maps of the Ventral–Lateral Region

Fate-mapping analysis in *Xenopus laevis* in the 32-cell stage has indicated that individual blastomeres can contribute to several organs, but that the position and the identity of the derived cells are highly reproducible, so that a fate map of the early embryo can be constructed (Wetts and Fraser, 1989; Moody, 1987; Dale and Slack, 1987a). In vertebrates other than amphibians, the fate of individual blastomeres is affected by more extensive cell mixing (in zebrafish, see Helde *et al.*, 1994; for fate maps of zebrafish, see Kimmel *et al.*, 1990, but see Strehlow *et al.*, 1994; for chick, see Hatada and Stern, 1994; for mouse, see Lawson *et al.*, 1986, 1991; Smith *et al.*, 1994; Beddington and Robertson, 1999).

Fate maps and molecular manipulations have suggested that, at least in *Xenopus*, there is a link between the specification of dorsal and anterior structures, on one hand, and ventral and posterior structures, on the other. This is evidenced in experimentally perturbed embryos. UV-irradiation of the vegetal pole prior to the first cell cycle ventralizes the embryo by blocking cortical rotation (Malacinski *et al.*, 1975). The effect of UV irradiation on dorsal structures also affects anterior development. The longer the exposure to UV irradiation, the more progressive loss of anterior structures, suggesting that the development of the dorsal and anterior programs are linked mechanistically. At the other end of the spectrum, exposure of embryos to lithium treatment at the 16- or 32-cell stage results in completely dorsoanteriorized embryos, and a loss of ventral and posterior structures (Klein and Melton, 1996). Based on these observations, a dorso-anterior index (DAI) has been established (Kao and Elinson, 1988; see Fig. 2). The effect of lithium on posterior and ventral fates is also observed in the fish, where the linkage between the dorso-anterior axis is also manifested in lithium-treated embryos (Stachel *et al.*, 1993).

A late blastula *Xenopus laevis* fate map (Fig. 1; based on Keller, 1975, 1976) shows that the ventral and posterior domains are largely overlapping in all three germ layers. In the ectoderm, anterior neural and non-neural fates are specified at a neural–epidermal boundary, with more posterior fates being specified outwardly from this junction (Fig. 1). In the mesoderm, fate maps have revealed the presence of at least two subdomains along the animal/vegetal pole axis, both in cell fate and morphogenetic movements. The marginal zone subdomain located toward the animal pole gives rise mostly to muscle, and cells located in this domain show convergent extension movements. The subdomain closer to the vegetal pole gives rise to blood, and is termed the leading edge mesoderm (Fig. 1; see Keller, 1975, 1976). Interestingly, cells that give rise to somitic mesoderm, defined traditionally as a dorsal structure, also originate from the ventral domain of the marginal zone. However, more anterior somites are specified closer to the dorsal lip, and posterior ones in the ventral side, suggesting that the anteroposterior and dorsoventral axis are linked. The pattern of formation of the blood islands (classically the ventral-most mesoderm) in the leading edge mesoderm makes us question how dorsoventral fates are specified, since some blood islands originate from cells closer to the dorsal lip (Fig. 1; Lane and Smith, 1999). These observations have prompted embryologists to reevaluate the fate maps and have generated some debate about how early dorsoventral cell fate specification is established. Recent fate-mapping analysis of the *Xenopus* endoderm has supported the notion that, in this layer, the dorsal endoderm is fated to give more anterior structures, and the ventral endoderm more posterior structures, again suggesting that the axes are linked for all three layers (Fig. 1; Chalmers and Slack, 2000). However, although fate maps have been very informative, they do not address how molecular mechanisms and morphogenesis affect cell fate specification. Ideally, we would like to correlate the fate maps with gene expression maps (and eventually protein expression maps) in order to understand how molecular events act to shape developmental decisions (see Vodicka and Gerhart, 1995). The development of gene expression maps has lacked behind the fate maps, and there is a need to

put them together in the context of the timing of developmental programs.

Establishment of Initial Ventral Fates in Amphibians

The mechanism of dorsoventral axis establishment is best understood in amphibians, where the initial asymmetry is established at fertilization by the sperm entry point. Sperm entry correlates with the future ventral domain of the embryo. This event promotes the process termed "cortical rotation," by which cortical cytoplasmic components rotate in relation to the inner endoplasm, in a process that is microtubule-dependent (Gerhart *et al.*, 1989). Cortical rotation appears to result in the nuclear accumulation of β-catenin, a component of the Wnt-signaling pathway (Larabell *et al.*, 1997; Fig. 3), and results in the establishment of the dorsal side. In other vertebrates, the establishment of the dorsoventral axis is less understood. In mice, the future dorsoventral axis is established during implantation (see Beddington and Robertson, 1999). In zebrafish, the initial cleavage planes are not aligned with the future dorsoventral axis (Helde *et al.*, 1994), and the timing of dorsoventral axis establishment is unclear. In chicks, the mechanisms underlying the initial D/V axis establishment are unknown.

Three-Signal Model of Mesoderm Induction

Mesoderm arises from the marginal zone as a result of inductive interactions between cells in the animal and vegetal poles. Nieuwkoop (1969a) demonstrated that the endoderm induces mesoderm formation through an interaction with the ectoderm during cleavage stages. Dorsal vegetal cells induce dorsal mesoderm (notochord and muscle), and ventral vegetal cells induce ventrolateral mesoderm (mesenchyme, blood, and some muscle). Transplanted dorsal vegetal blastomeres can induce axial structures, and they have been termed the "Nieuwkoop center" following Nieuwkoop's work (see Kessler and Melton, 1994). The Nieuwkoop center induces the mesodermal, or Spemann's, organizer, which is required for the patterning of all three germ layers (for a review on the organizer, see Harland and Gerhart, 1997).

Several lines of evidence have led to the proposal of the three-signal model of mesoderm induction (Smith, 1989; reviewed in Kessler and Melton, 1994; Harland and Gerhart, 1997). In this model, the endoderm blastomeres release two signals; first, a ventralizing signal that induces ventral mesoderm (lateral plate, mesenchyme, and blood) all throughout; and, a second signal originating from the dorsal endoderm (Nieuwkoop center) that induces the formation of the organizer in the dorsal mesoderm. Signal 1 is localized to the vegetal pole of the embryo. There has been considerable debate as to whether this signal is maternal or zygotic in origin. However, it appears that induction of the Nieuwkoop center is highly regulated by zygotically expressed genes (Agius *et al.*, 2000). Signals 1 and 2 need not be different in nature. The third emanates from the organizer, and is able to dorsalize all three germ layers. The elucidation of the molecular nature of these signals has been the focus of intense scrutiny over the last few years, although the evidence suggests that signals 1 and 2 are members of the TGF-β family, most likely nodal-related members, and signal 3 is likely mediated by BMP-inhibitory molecules (see discussion below). In this review, we will focus mostly on current models for mesendoderm regionalization rather than on its induction.

Ventral and Posterior Specification of the Ectoderm

Epidermal versus neural fate specification. The ectoderm gives rise to epidermis in the ventral side and to neural tissue in the dorsal side. Currently, the view for neural specification in vertebrates is that, in the absence of BMP signals, ectodermal cells will differentiate as neural, in a model that has been termed "the default model" for neural induction (reviewed in Weinstein and Hemmati-Brivanlou, 1999). Ectodermal cells located adjacent to the organizer are exposed to direct BMP inhibitors, such as chordin, noggin, follistatin, and cerberus, which dorsalize all three germ layers and therefore induce neural cell fates in the ectoderm (Fig. 3). The organizer also secretes the nodal-related factor Xnr-3, which appears to block BMP signaling, although its mechanism of action is unclear (Hansen *et al.*, 1997). While BMP inhibitors induce telencephalic fate, factors such as FGFs, Wnts, and retinoic acid have been shown to caudalize neural tissue to generate more posterior structures (midbrain, hindbrain, and spinal cord). For reasons of space and also because neural induction and patterning has been extensively reviewed, we will not discuss the posterior fate determination in the nervous system any further (for a review, see Weinstein and Hemmati-Brivanlou, 1999).

Ventral Specification in the Ectoderm: Role of Bone Morphogenetic Protein (BMP) Signaling

In cell dissociation experiments with *Xenopus* animal cap cells, BMP signaling has been shown to modulate the fate of competent gastrula ectodermal cells. No BMP signaling leads to neural induction (Weinstein and Hemmati-Brivanlou, 1999), whereas low BMP signaling leads to the induction of intermediary fates, such as cement glands, sensory placodes, and neural crest cells, and high BMP signaling leads to induction of epidermal fates (Wilson and Hemmati-Brivanlou, 1997). The effects of BMPs and GDFs (growth and differentiation factors, TGF-β members which signal through BMP-receptors) on epidermal development have been well characterized and the molecules that mediate the effects of BMPs in this system are fairly well understood. Despite the relatively recent discovery of BMP activities, the biochemical pathway has been resolved to a

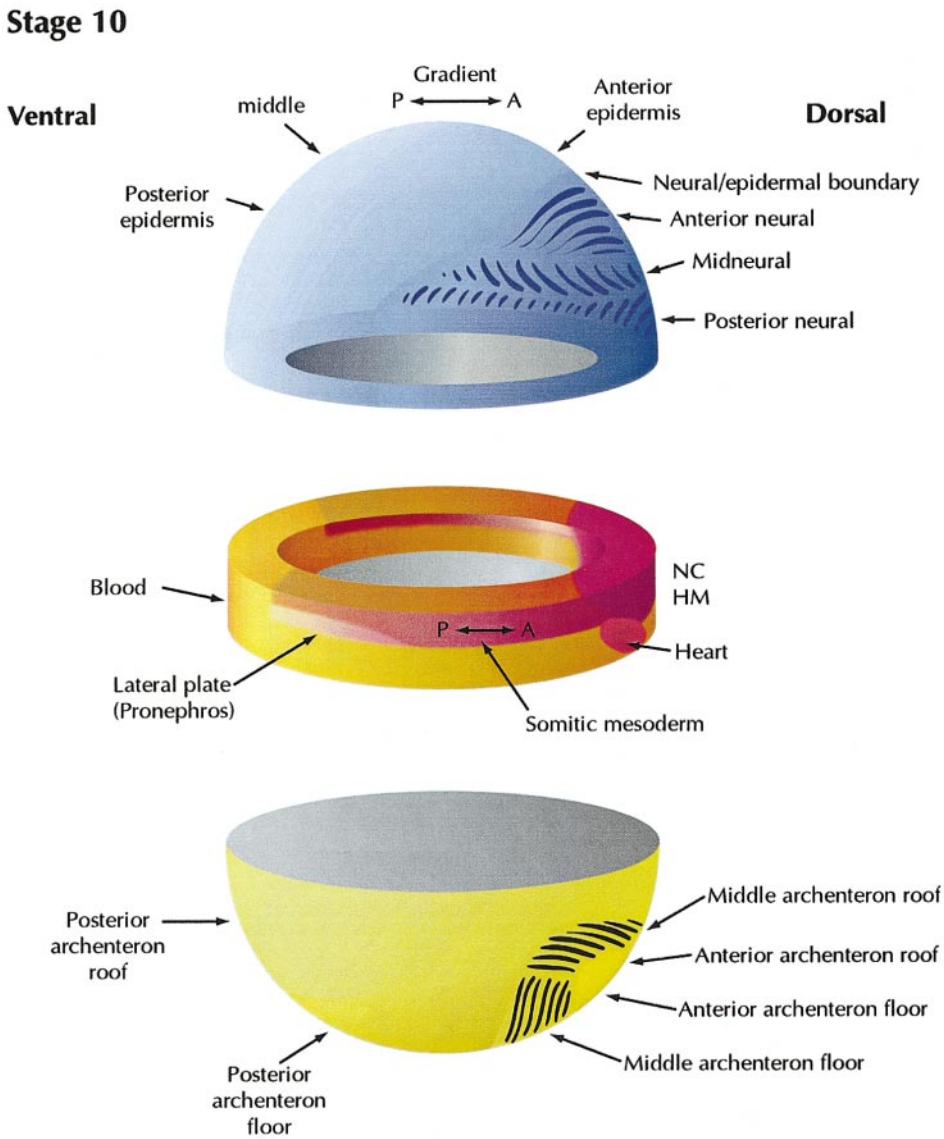

FIG. 1. Fate map of the late blastula *Xenopus laevis* embryo. Schematic diagram of a *Xenopus laevis* late blastula (based on the work of Keller, 1975, 1976) prior to formation of the bottle cells and the dorsal lip. Fate-mapping analysis has shown that the three germ layers show patterning along the anterioposterior and dorsoventral axis. The domains fated to give rise to ventral and posterior cell fates are highlighted. The ectoderm originates mostly from the animal pole (upper panel). The non-neural, epidermal ectoderm (blue) originates from the ventral two-thirds of the animal pole. The neural ectoderm (striped) originates from the ectoderm overlaying the dorsal lip. Notice that the anterior domains of both neural and non-neural ectoderm form adjacent to each other at the neural/epidermal boundary, where neural crest cells develop. The origins of this boundary and the presence of patterning signals in this boundary are unknown. The mesoderm originates from the marginal zone (MZ; middle panel). The dorsal end is determined as the place where the dorsal lip will form. In the dorsal marginal zone (DMZ), the notochord (NC) and head mesoderm (HM) form most dorsally (purple). Along the MZ, tissues derived in a dorsoventral gradient are: paraxial mesoderm (muscle), heart mesoderm, lateral plate mesoderm (pronephros), and blood (ventral-most mesoderm; yellow). Notice that the ventral marginal zone (VMZ) encompasses 300° in the radial embryo, whereas the DMZ only 60°. Fate maps have revealed that, along the anteroposterior axis, the somites, a dorsal mesodermal derivative, also originate from the ventral marginal zone. A minor percentage of the blood islands, a ventral-most derivative, originate from the DMZ, close to the organizer (see text for details). The endoderm originates exclusively from the vegetal pole (bottom panel). Image shows the fate map of the superficial endoderm. No fate maps are available from the deep endoderm. Notice how the posterior archenteron develops from mostly ventral endoderm. The anterior and middle archenteron develops from the dorsal endoderm (yellow and striped pattern), reinforcing the notion that there is a linkage of the ventroposterior and dorsoanterior axis in all three germ layers.

FIG. 2. Experimentally perturbed *Xenopus* embryos and the dorsoanterior index (DAI). Varying amounts of exposure to UV irradiation during the first cell cycle yields embryos that are increasingly ventroposteriorized (DAI of 0, most ventroposteriorized, to DAI of 5, untreated embryo). Exposure to LiCl for varying times yields embryos progressively more dorsoanteriorized (DAI of 10, most dorsoanteriorized). At the extremes of the DAI index, an embryo with a DAI of 0 lacks the head and axial structures entirely. An embryo with a DAI of 10 contains only radially symmetrical head structures. These experimentally disturbed embryos, and the DAI index, illustrate that the ventral and posterior axis, on the one hand, and the dorsal and anterior axis, on the other, are linked in vertebrates.

large extent (see Fig. 3; for a review of TGF-β signaling, see Massague and Chen, 2000). The involvement of BMPs in ectodermal fates has been supported by studies in zebrafish mutants affecting the BMP pathway, such as *swirl/bmp-2* (Nguyen *et al.*, 1998) or in studies with exogeneous BMPs (Neave *et al.*, 1997). In the induction of ventral cell fates in *Xenopus* during gastrulation, BMP-4 appears to act as a morphogen, inducing distinct cell fates at different thresholds. While a gradient of BMP protein or activity has not been demonstrated in the embryo, it does provide a working model as to how patterning along the D/V and A/P axis could take place. In theory, the graded distribution of BMPs in the gastrula ectoderm can act to instruct different fates, with lowest levels in the future dorsal neural plate, and highest levels in the future epidermis, at a distance from the organizer. One hypothesis as to how BMP signaling gradients might be established across the ectoderm is that cells are exposed to different concentrations of BMP signals and for different lengths of time (Wilson and Hemmati-Brivanlou, 1997; in zebrafish, see Barth *et al.*, 1999). Since BMPs are presumed to act non-cell-autonomously at short distances *in vivo* (Nikaido *et al.*, 1999), the effective response of cells to BMP gradients may be modulated by diffusible antagonists secreted by the organizer, which may act to decrease the concentration and time of exposure of ectodermal cells to BMPs (see Barth *et al.*, 1999 for elegant work in the zebrafish), a mechanism seemingly conserved across species for fate specification in the ectoderm.

In fish, it appears that modulations in BMP levels affect fate specification along the D/V axis, without affecting the identity of those cells along the A/P axis (Barth *et al.*, 1999). Therefore, BMP signals might be used in ventral fate acquisition, but not posterior identities in the ectoderm. In *Xenopus*, the effect of different concentrations of BMPs on ectodermal fates can be mimicked by exposure of ectodermal cells to different concentrations of the signal transducers Smad1 and Smad5 (Wilson and Hemmati-Brivanlou, 1997; Suzuki *et al.*, 1997a), suggesting that the morphogenic effects of BMPs in ectodermal fates may be modulated at the level of transcriptional regulation of gene expression. The ultimate decision of an ectodermal cell to become epidermal or neural, according to its position along the D/V axis, might be dependent on the final integration of the initial BMP signal. Regulation of BMP signaling is modulated extracellularly by inhibitors secreted from the organizer, at the cell surface (such as by the truncated type-I receptor BAMBI), and intracellulary by inhibitors, such as Smad-6, Smad-7, and Smurfs (see Massague and Chen, 2000). It is ultimately the levels of activated Smad-1 and Smad-2 which will transduce a signal on behalf of BMPs or TGF-β molecules (which compete for binding and signaling through Smad-4), and will influence the decision of a cell to adopt its fate. For this reason, in order to understand signaling events in the context of epidermal patterning, we must gain insights into the final readout of these complex signaling pathways in the context of different positions along the D/V and A/P axis.

Msx genes have been implicated in epidermal development, since their expression is directly regulated by BMP signaling in *Xenopus* (Suzuki *et al.*, 1997b). Msx-1 is a direct early induced gene that can block neural induction by dominant negative BMP-Rs. When overexpressed in dissociated ectodermal cells, which would normally give rise to neural tissue, Msx-1 expression can induce the formation of epidermal fates (Suzuki *et al.*, 1997b). In addition, the *Xenopus* translation initiation factor 4AIII (eIF-4AIII) has also been implicated in epidermal fates. eIF-4AIII has been shown to induce epidermal fates in dissociated cap cells, indirectly activating the BMP-Receptor via a soluble factor of unknown identity (Weinstein *et al.*, 1997). The involve-

ment of eIF-4AIII in epidermal fate acquisition highlights the possible modulation of ectodermal fates by post-transcriptional mechanisms. The effect of post-transcriptional regulation of gene expression is an area that deserves a closer scrutiny in the light of developmental decisions.

Posterior Specification in the Epidermis

Whereas a wealth of information exists about the molecules implicated in anterior–posterior patterning of the neural ectoderm, the regionalization of the epidermal ectoderm has been largely ignored. This is partly due to the lack of morphological features that distinguish different domains of the early non-neural ectoderm. There are clear distinctive regional features in the epidermal ectoderm in different species, and the molecular mechanisms that lead to their specification have been studied. For instance, in *Xenopus*, low levels of Bmp signaling lead to formation of the cement gland in the anterior ectoderm (see Gammill and Sive, 2001; and references within) and the Notch-Delta lateral-inhibition pathway operates for the formation of ciliated cells posterior to the cement gland in *Xenopus* ectoderm (Deblandre *et al.*, 1999) and in the formation of the lateral line placodes of *Xenopus* and fish embryos (see Winklbauer, 1989; Schlosser and Northcutt, 2000). However, the early cues that prepattern the non-neural ectoderm are largey unknown, and this is an area that is in need of further investigation.

Several ectodermal markers show restricted expression along the anterior–posterior axis in the epidermis in vertebrates. Several lines of evidence suggest that nonaxial signals act to pattern the non-neural ectoderm (Read *et al.*, 1998; Yamada *et al.*, 1999). Among the genes studied in early A/P patterning in the epidermis is the *Xenopus* epsin (Xepsin) gene, a serine-protease with no known orthologs in other species. Xepsin transcripts are localized to the anterior non-neural ectoderm, and its transcriptional regulation has been used to study A/P patterning in the epidermis. Xepsin expression can be inhibited in recombination experiments between anterior ectoderm and ventral or lateral marginal zone explants, suggesting that there is an active mechanism which restricts Xepsin expresion to the anterior epidermis (Yamada *et al.*, 1999). Xepsin expression is inhibited by exposure to retinoids or by injection of a constitutively active retinoic acid receptor (Yamada *et al.*, 1999), suggesting that retinoic acid signaling regulates Xepsin expression. However, a dominant negative retinoic acid receptor does not expand its expression posteriorly, suggesting that if retinoids are involved in the repression of posterior expression of Xepsin, additional factors may be required (Yamada *et al.*, 1999). Presently, it is unclear whether other factors involved in posteriorizing the neural ectoderm, such as FGFs and Wnts, play a role in restricting Xepsin expression to the anterior epidermis. Studies with HoxD1 and Xgbx2, genes expressed at similar positions in the neural and non-neural ectoderm, suggest that common mechanisms may regulate the A/P expression of these genes in the neural and epidermal ectoderm (Kolm and Sive, 1995; von Bubnoff *et al.*, 1995). Studies in both *Xenopus* and zebrafish have supported a model where FGFs and Wnts can act to posteriorize non-neural ectoderm as well, as evidenced from the regulation of the expression of GATA-2 and -3 genes in non-neural tissues (Read *et al.*, 1998).

However, studies with several homeobox genes in ascidian embryos suggest that, at least in this system, the regionalization of the neural and epidermal ectoderm may be determined through distinct molecular mechanisms (Wada *et al.*, 1999). The anterior and posterior epidermal domains may be specified independently, and not by conversion of the anterior into posterior by caudalizing molecules (Wada *et al.*, 1999). Whether similar patterning mechanisms operate in non-neural and neural ectoderm along the A/P axis deserves closer attention. In addition, the importance of both vertical and planar signals in the regulation of the polarity of the epidermal ectoderm awaits further elucidation, as is the characterization of more markers with regional expression. It is of interest to characterize more regional epidermal markers in order to address the role of axial and nonaxial signals in the specification of distinct fates in the epidermis. It is presently unclear whether the putative gradient of BMP inhibitors secreted from the organizer also plays a role in fate specification in the epidermis, particularly at the junction between the neural and non-neural ectoderm. Finally, there is a need to understand what this regionalization means in the context of epidermal cell fates in different vertebrate species.

Inductive Signals Involved in Ventral and Posterior Specification of the Mesoderm

Nieuwkoop demonstrated that there are preexisting dorsoventral differences in the endoderm that promote particular mesodermal fates. For example, when dorsal endodermal cells are cultured with ectoderm, only dorsal mesoderm forms, whereas ventral endodermal cells and ectoderm form only ventral mesoderm. (Nieuwkoop, 1969b; Dale and Slack, 1987b). Furthermore, in UV-irradiated embryos, only ventral mesoderm forms, suggesting that the formation of the organizer acts to regionalize the mesoderm. The ectoderm derived from wild-type blastula in these recombination experiments also has some dorsoventral prepattern, since dorsal versus ventral ectoderm gives rise to different mesodermal fates in the presence of exogenous mesoderm inducers (Bolce *et al.*, 1992). Interestingly, some blood island precursors arise from the organizer region in hyperdorsalized embryos exposed to lithium, suggesting that our understanding of the dorsoventral patterning of the marginal zone needs to be reevaluated (Lane and Smith, 1999; Kumano *et al.*, 1999). Below, we will describe the main signaling pathways that affect the development of ventral and posterior mesoderm, and we will also highlight the evidence for the involvement of several transcription factors implicated in ventral and posterior mesoderm fate specification.

BMP Signaling in Ventral and Posterior Cell Fate Determination in the Mesoderm

A crucial step toward dorsoventral polarity establishment is the induction of the organizer region. BMPs have been shown to suppress the maintenance of the organizer and therefore act to ventralize the *Xenopus* embryo (Jones *et al.*, 1996b). Overexpression of BMPs phenocopies the most severe effects of UV-irradiation, although the mechanism of action of BMPs and UV-irradiation is likely a distinct process. The inhibition of organizer formation by BMPs is due to the loss of expression of organizer genes (Laurent and Cho, 1999). The antagonistic effect of activin/Vg1 and BMPs on organizer gene expression may be mediated through competition for available pools of SMAD-4 protein, the signal transducer implicated in both signaling pathways (Fig. 3; Massague and Chen, 2000). Several components of the BMP pathway have been shown to play a critical role in posterior and ventral patterning of the mesoderm.

Among the Bmp ligands, Bmp-2, Bmp-4, and Bmp-7 have been implicated in posterior and ventral fate specification in vertebrates, although their expression patterns and loss-of-function phenotypes can differ across the species studied. Bmp-4 has been strongly implicated in ventroposterior development in *Xenopus*, zebrafish, and mouse. Its expression patterns, as well as results from loss- and gain-of-function experiments in zebrafish and *Xenopus*, suggest that Bmp-4 is a strong candidate to be a morphogenic determinant of ventral and posterior fates (Dale *et al.*, 1992; Jones *et al.*, 1992, 1996b; Nikaido *et al.*, 1999;). Loss of BMP-4 function in the mouse yields severe defects in the hematopoetic system and posterior region of the embryos (Winnier *et al.*, 1995), consistent with the results obtained in zebrafish and *Xenopus*, where overexpression of Bmp-4 causes an expansion of ventral and posterior structures (Dale *et al.*, 1992; Jones *et al.*, 1994; Neave *et al.*, 1997). Although no Bmp-4 loss-of-function mutations have been identified in the zebrafish, it has been proposed that the zebrafish Bmp-2 gene is functionally equivalent to the *Xenopus* and mouse Bmp-4 genes (Kishimoto *et al.*, 1997).

Loss of Bmp-2 function in the *swirl* mutation in the zebrafish leads to a phenotype similar to that of the mouse Bmp-4 loss-of-function (Mullins *et al.*, 1996; Kishimoto *et al.*, 1997), and it demonstrates that Bmp-2 acts endogeneously to promote ventral fates. Whereas Bmp-2 and Bmp-7 have been strongly implicated in the establishment of ventral and lateral fates in zebrafish (Kishimoto *et al.*, 1997; Dick *et al.*, 2000; Schmid *et al.*, 2000), since loss of these genes in the *swirl* and *snailhouse* zebrafish mutants, respectively, leads to a severe dorsalization phenotype, these genes appear not to have redundant functions in the zebrafish embryo, and therefore they may act as heterodimers in ventral fate specification (Schmid *et al.*, 2000). However, loss of Bmp-2 or Bmp-7 function in the mouse does not lead to defects in D/V patterning (Zhang and Bradley, 1996; Dudley *et al.*, 1995; Luo *et al.*, 1995). Redundancy in BMP function and the role of maternal versus zygotic BMP activities in ventral fate specification may account for the lack of early patterning defects in mice lacking BMP functions, and multiple genetic combinations might be required to show a role for these genes in ventral patterning in mice. In zebrafish, a mutation in the *Alk-8/lost-a-fin* gene leads to embryos dorsalized in a fashion similar to loss-of-function of *bmp-2/swirl*, *bmp-7/snailhouse* embryos. Alk-8 is a type-I BMP-receptor ubiquitiously expressed during the gastrulating zebrafish (Mintzer *et al.*, 2001; Bauer *et al.*, 2001). Whether all BMP signaling in ventral fate specification in zebrafish (and other vertebrates) signals through Alk-8, or also through other type-I BMP receptors expressed at the relevant stages, such as Alk-6, remains to be elucidated.

Downstream from the initial signaling, the BMP-signal transducers Smad-1 and Smad-5 appear to mediate Bmp-signals in ventral fate specification. The zebrafish dorsalized mutant *somitabun* results from a missense mutation in the Smad-5 gene, which appears to act in a dominant negative fashion (Hild *et al.*, 1999). Although this mutation implicates Smad-5 as an endogenous mediator of Bmp-signaling in ventral fates, multiple results suggest that this is unlikely to be the case prior to gastrulation. First, the *somitabun* mutation can block the function of both Smad-5 and Smad-1, and the mutation can be rescued by Bmp-2/4 RNA injections (Hild *et al.*, 1999), suggesting that these ligands signal through another Smad signal transducer. This is most likely Smad-1, since Smad-1, but not Smad-5, can rescue the *swirl/bmp-2* loss-of-function mutants (Dick *et al.*, 1999). Furthermore, Smad-1 expression is itself regulated by Smad-5 and Bmp-2b in zebrafish (Dick *et al.*, 1999), which could explain the dorsalization phenotype in the *somitabun* mutant. In this mutant, expression of Bmp-2 is decreased, as Bmp-2b signaling enhances its own expression (Hild *et al.*, 1999). Therefore, a role for Smad-5 itself in zebrafish ventroposterior development will have to wait for loss-of-function mutations. However, the expression patterns of Smad-1 and Smad-5 in different species varies, and so it is likely that they carry out distinct functions during embryogenesis (Dick *et al.*, 1999). In *Xenopus*, there is maternal Smad-1 contribution (Graff *et al.*, 1996), whereas, in the zebrafish, Smad-1 is not expressed until midgastrulation (80% epiboly; Hild *et al.*, 1999), whereas Smad-5 is expressed at all stages. Further analysis will clarify the roles of these and other molecules in mediating Bmp-signals in ventral and posterior development.

Wnt Signaling in Initial Ventral Fate Specification

The role of Wnts on D/V axis establishment has been intensively studied (Moon *et al.*, 1997). Wnts are able to induce secondary axis if expressed vegetally prior to the midblastula transition (MBT), although Wnts can promote ventral and posterior fates if expressed after MBT (Christian and Moon, 1993). It is of interest to understand the biochemical changes at MBT that modify Wnt activities in

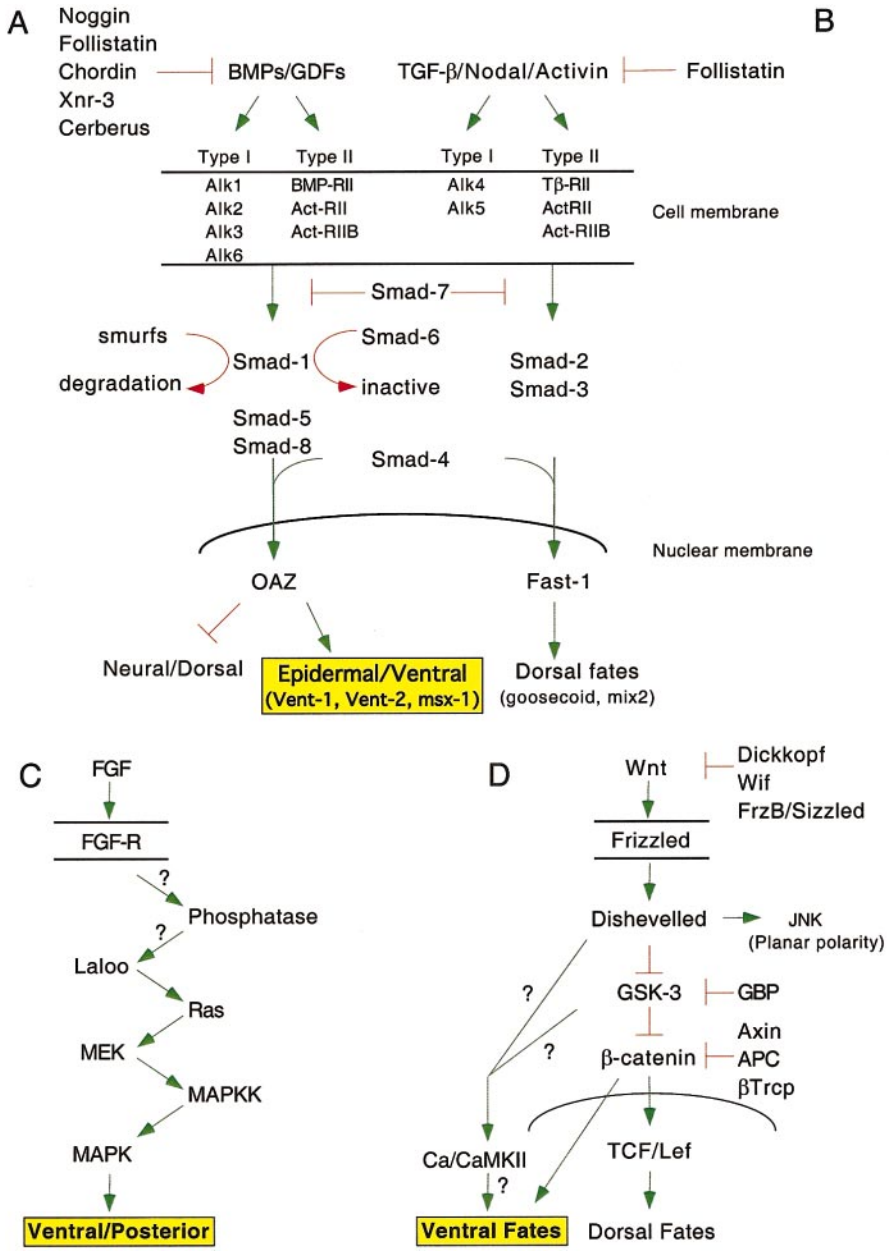

FIG. 3. Signaling pathways implicated in ventral/posterior fate specification in *Xenopus laevis.* The four major signaling pathways implicated in ventral and posterior fate specification are shown. (A) BMP/GDF pathway; (B) TGF-*β*/Activin/Nodal pathway; (C) FGF pathway; (D) Wnt pathway. In the case of the Activin/Nodal pathway, a graded response to these molecules acts to pattern the mesoderm along the dorsoventral and anteroposterior axis. Highest dose of these molecules results in more dorsoanterior fates, and therefore the figure indicates their effect in the dorsal pathway. For a discussion on the role of the TGF-*β*, nodal and activin pathways in ventral and posterior fates, see the text. Please see the text for a discussion on the specific involvement of each signaling pathway in the specification of ventral and posterior fates in each of the three germ layers. Green arrows indicate stimulatory regulators, red arrows inhibitory regulators of the signaling pathways. Question marks indicate our lack of knowledge of the molecules implicated in each step.

mesodermal fate specification. The role of Wnts on the dorsal side of the marginal zone has been under closer scrutiny, and the Activin/Vg1 and the Wnt pathways have been shown to positively and cooperatively regulate/induce organizer genes, such as goosecoid and chordin (Crease *et al.*, 1998; Harland and Gerhart, 1997, and references within). The role of Wnts in dorsal axis formation and neural patterning has been confirmed in mice null for

Wnt3a (Liu *et al.*, 1999), although no mutations in the Wnt signaling pathway have been described that affect early posterior and ventral patterning. The signaling pathway initiated by Wnt signals in dorsal fate specification is referred to as the canonical Wnt pathway, which is β-catenin-dependent (Fig. 3; Peifer and Polakis, 2000). In contrast, the ability of Wnts to induce ventralization phenotypes is poorly understood, although it appears to follow a β-catenin-independent mechanism (Fig. 3; Kuhl *et al.*, 2000). Presently, there are two distinct Wnt signaling pathways that diverge at the level of dishevelled (Tada and Smith, 2000; Fig. 3). Wnt-1, -3A, -8a, and -8b belong to the first class, and Wnt-4, -5a, -7a, and -11 belong to the second class (Kuhl *et al.*, 2000; Tada and Smith, 2000; Fig. 3).

Exposure of embryos to Wnts after MBT leads to a similar ventralization phenotype to that observed when embryos are exposed to lithium at the onset of gastrulation, which leads to a progressive loss of anterior structures. This effect is strongest immediately after MBT, and decreases progressively throughout gastrulation (Fredieu *et al.*, 1997; Yamaguchi and Shinagawa, 1989). One target of lithium is GSK-3, a negative regulator of Wnt signaling, therefore implicating GSK-3 in the establishment of ventral fates as well. This is a bit of a paradox, since GSK-3 inactivation is expected to stabilize β-catenin, therefore activating the canonical Wnt pathway, although at least one mechanism of action of Wnts in promoting ventral fates appears to be independent of β-catenin (Kuhl *et al.*, 2000). It is possible that both β-catenin-dependent and -independent mechanisms operate in the establishment of ventrolateral fates.

Overexpression studies with Xwnt-5a and Xwnt-11 have shown that these Wnts do not induce secondary axis in *Xenopus* (Kuhl *et al.*, 2000). Although this might be due to the inability of these Wnts to bind endogenous *Frizzled* (Fz) receptors, these Wnts can block dorsal marker induction by Xwnt-8 RNA injections, suggesting that they signal through a distinct mechanism that may compete for available components. Furthermore, these Wnts may be implicated in ventral fate development, since a dominant negative mutant of Xwnt-11 induces some dorsal markers and inhibits vent-1 expression (a ventral mesoderm marker; Kuhl *et al.*, 2000). However, overexpression of Xwnt-5a or Xwnt-11 does not lead to a ventralization phenotype, and therefore their role in mediating ventral fates is uncertain at the moment (Kuhl *et al.*, 2000). It appears that signaling through these Wnts may be mediated by Ca^{2+}-signaling and Ca^{2+}-calmodulin-dependent kinase-II (CaMKII; Kuhl *et al.*, 2000), in a β-catenin-independent pathway. However, at which point the Wnt-signaling pathways diverge upstream of β-catenin and the players involved in ventral fate specification are unknown. The results from exposure to lithium strongly suggest that GSK-3 is implicated (Klein and Melton, 1996), which is different from the role of Wnt signaling in gastrulation movements in *Xenopus* and zebrafish, also mediated by Wnt-11, in a GSK-3- and β-catenin-independent pathway (Tada and Smith, 2000; Heisenberg *et al.*, 2000). Interestingly, the activity of Wnts in the marginal zone might be regulated at the level of secreted inhibitory molecules, such as *frzb* in the organizer region (Wang *et al.*, 1997) and *sizzled* in the ventral-most marginal zone (Salic *et al.*, 1997). The expression pattern of these molecules in the marginal zone argues for at least four regions of distinct Wnt signaling within the marginal zone, suggesting that patterning of the ventral marginal zone along the equatorial axis may be specified by signals from both ends of the equatorial region. The effect of Wnt signals in fate specification must come from a local integration of the inputs from the different inhibitors and both Wnt pathways acting in the marginal zone.

Nodal Signaling in Ventral and Posterior Mesoderm Development

Genetic studies have strongly implicated the nodal-related genes in mesendoderm formation in both mouse and zebrafish (reviewed in Schier and Shen, 2000), and to date nodal-related factors remain the most likely candidates to mediate endogeneous mesendoderm induction. Six nodal-related genes have been identified in amphibians, Xnr-1, -2, -3 and -4, -5, and -6 (Schier and Shen, 2000; Takahashi *et al.*, 2000). Xnr-3 appears to have different biochemical activities from the other Xnrs (Hansen *et al.*, 1997). Nodal-related genes appear to signal through type-II activin receptors, although specific type-I receptors have not been identified yet (Fig. 3). In zebrafish, all axial mesoderm is lost in double mutants of the nodal-related genes *cyclops* and *squint* (Feldman *et al.*, 1998), and mice lacking Nodal lack the primitive streak (Conlon *et al.*, 1994). Recent studies suggest that these genes may be required for the allocation of dorsal marginal cells to form mesendoderm, since the involution movements of mesendodermal cells are also blocked in these mutants (Feldman *et al.*, 2000). Studies in zebrafish suggest that higher level nodal signaling is required for anterior axial mesoderm specification than for the posterior mesoderm (Gritsman *et al.*, 2000). In the mutant *one-eye-pinhead*, an EGF-CFC member required for nodal signaling, the endoderm, the prechordal plate, and the notochord are missing (Schier *et al.*, 1997; Gritsman *et al.*, 1999). Since the phenotype of maternal and zygotic null for *one-eye pinhead* is identical to double homozygotes for *cyclops* and *squint* (Gritsman *et al.*, 1999), it is likely that *one-eye-pinhead* is required for nodal signaling in zebrafish mediated by these ligands. Whether other nodal signaling events occur in the absence of these genes remains to be addressed. However, these results have to be carefully evaluated, since a report strongly suggests that *one-eye pinhead* is also implicated in BMP-signaling (Kiecker *et al.*, 2000). Furthermore, a mouse null for an EGF-CFC member, *cripto*, leads to embryos lacking embryonic mesoderm and all posterior structures, and might be required for the cell movements necessary for proper A/P axis establishment (Ding *et al.*, 1998).

Activin and FGF Pathways in Mesoderm Induction and Patterning

There has been some discrepancy in the literature as to whether the mechanism for mesoderm development is based on a direct morphogenetic effect of activin or BMPs, or on a relay mechanism. These models are important in our understanding of how morphogens can pattern the mesoderm along its entire dorsoventral as well as anterior–posterior axis. Experimental evidence suggests that both mechanisms may be at play. Activin has been shown to act as a morphogen, inducing different fates at different thresholds (Jones *et al.*, 1996a; McDowell *et al.*, 1997), although a relay model initiated by activin exposure has also been supported in animal cap experiments (Reilly and Melton, 1996; Rodaway *et al.*, 1999). Regardless of the mechanism of diffusion, it has been clearly established that activins (just like BMPs) can act as morphogens, eliciting different fates at different thresholds of activities. Thus, exposure of cells to the highest dose of activin leads to endoderm fate specification. Within the mesoderm, the lowest dose of activin activity elicits ventral and posterior fates and the highest dose induces the expression of dorso-anterior fates (Green and Smith, 1990; McDowell *et al.*, 1997). Although mice null for zygotic activins do not have defects in mesoderm formation or patterning (Matzuk *et al.*, 1995), it is possible that there is a role for maternal activins in mesoderm formation. Mice null for both type-II activin receptors have been generated, supporting a role for these partially redundant receptors in mesoderm formation (Song *et al.*, 1999). Since the type II receptors can transduce signals on behalf of activins and nodals, and although the current model presents nodal signaling as the main mediator of mesoderm induction, it is likely that proper patterning along the A/P axis requires both the nodal and activin pathways (see Thisse *et al.*, 2000 for nodal and activin signaling in A/P axis formation in the zebrafish).

There is some evidence that bFGF is not required for initial mesoderm induction, but that it is required to maintain mesodermal fates (Isaacs *et al.*, 1994; Schulte-Merker and Smith, 1995). FGF signaling has been shown to be critical for trunk and tail mesoderm development in *Xenopus* (Amaya *et al.*, 1991) and zebrafish (Griffin *et al.*, 1995), since blocking FGF-signaling leads to defects in posterior mesoderm formation, and FGFs have been shown to induce expression of Brachyury in *Xenopus* and zebrafish (Schulte-Merker and Smith, 1995; Griffin *et al.*, 1995). Dong *et al.* (1996) have reported on the role of the AP-1 transcription factor in FGF-mediated posterior and lateral mesoderm formation, since blocking AP-1 function in embryos results in posterior truncation resembling loss of FGF signaling. AP-1 appears to function downstream of the FGF-Ras-Raf-MAPK pathway in mesoderm induction, but not in activin-mediated mesoderm induction (Fig. 3). Laloo, a Src-kinase homolog, induces posterior axis duplication and mesoderm induction when overexpressed in *Xenopus* embryos (Weinstein *et al.*, 1998). Laloo acts downstream of the FGF receptor and upstream of Ras in the mesoderm induction pathway (Fig. 3). A dominant negative form of Laloo is able to block mesoderm induction in animal caps exposed to FGF and activin (Weinstein *et al.*, 1998). The involvement of endogeneous laloo in mesoderm formation is demonstrated by the effect of dominant negative laloo in blocking mesoderm formation in the embryo. However, the endogeneous phosphatases that mediate laloo dephosphorylation and activation, as well as the targets of activated laloo, are unknown.

Brachyury and Posterior Mesoderm Specification

Xenopus brachyury (Xbra), a pan-mesodermal T-box gene, and orthologs are required for the formation of posterior mesoderm and notochord in mouse, zebrafish, and *Xenopus* embryos (Wilson *et al.*, 1995; Conlon *et al.*, 1996; Conlon and Smith, 1999; Halpern *et al.*, 1993; Herrman *et al.*, 1990). Elegant genetic studies done in mice lacking Bra (Wilson *et al.*, 1995) initially showed that brachyury is required for mesodermal cell movements through the primitive streak during gastrulation. In *Xenopus*, loss-of-function of Xbra leads to a loss of ventral and posterior mesodermal markers, and cells with reduced or lacking Xbra function undergo apoptosis, possibly as a consequence of a failure to undergo convergent extension in the absence of normal Xbra function (Conlon and Smith, 1999). Overexpression of *Xenopus* Xbra yields different effects on the type of mesoderm induced; low doses lead to ventral mesoderm specification, and higher concentrations yield to the induction of more dorsal mesoderm, such as muscle (Tada *et al.*, 1998). Xbra therefore also seems to have a morphogen-type activity, with a behavior similar to that of activins, and is clearly required for posterior and ventral fates. This suggests that the readout of an extracellular morphogen gradient can be mediated at the transcriptional level, as previously described for Smad1/5 (Wilson and Hemmati-Brivanlou, 1997; Suzuki *et al.*, 1997b) and Smad2 (Graff *et al.*, 1996). Bix-1, a target gene of Xbra function, is able to induce ventral mesodermal fates when overexpressed in the ectoderm and in the embryo, in a dose-dependent manner. High concentrations of Bix-1 downregulate Xbra expression and lead to the induction of endodermal markers in a cell-autonomous fashion (Tada *et al.*, 1998).

Another T-box gene, eomesodermin (*eomes*), has been shown to induce most mesodermal genes when overexpressed in *Xenopus* embryos (Ryan *et al.*, 1996), and it responds in a concentration-dependent fashion to different amounts of activin (Ryan *et al.*, 1996, 2000). *Eomes* clearly plays a role in mesoderm formation in both *Xenopus* (Ryan *et al.*, 1996) and mice (Russ *et al.*, 2000), where it is required for mesoderm formation and gastrulation movements (Russ *et al.*, 2000). However, its loss-of-function in the mouse does not yield any defects in the initial patterning along the anterior–posterior axis.

Role of VegT and Derriere in Ventral and Posterior Mesendoderm Patterning

The VegT T-box transcription factor is able to induce mesoderm and endoderm and its ablation from late blastula embryos inhibits mesoderm formation in animal caps (Zhang *et al.*, 1998; Casey *et al.*, 1999; Clements *et al.*, 1999). Similar roles have been ascribed to the zebrafish ortholog of VegT, *spadetail*, although in zebrafish, VegT is not maternally expressed (Kimmel *et al.*, 1989; Griffin *et al.*, 1998). Zebrafish *spadetail* mutations lead to a loss of trunk non-notochordal mesoderm (Kimmel *et al.*, 1989) and it likely functions, together with other T-box genes, downstream of FGF signaling in the specification of trunk and tail mesoderm (Griffin *et al.*, 1995, 1998; Amacher and Kimmel, 1998). In *Xenopus*, expression of the Bix4 gene rescues endoderm gene expression in VegT-depleted embryos, suggesting that this gene functions downstream of VegT in endoderm formation (Casey *et al.*, 1999). Mesoderm specification induced by VegT may be mediated through the activation of nodal-related signals and derriere. Derriere is a TGF-β family member implicated in the development of posterior mesodermal and ectodermal derivatives (Sun *et al.*, 1999). The effect of derriere overexpression is a reduction of anterior neural structures, which seems to result from a change of cell fates from anterior neuroectoderm to mesoderm. A dominant negative derriere (Cm-derriere), in which the processing site has been mutated, does not block the induction of mesodermal gene expression in animal caps by activin, Xnr-2, -3, and -4 genes, or Vg1 (Sun *et al.*, 1999), suggesting that it is acting upstream or that it is not required for this induction. Experiments with the Cm-derriere in whole embryos support the notion that derriere is required for posterior mesoderm development, and suggests that a feedback loop may be formed between VegT and derriere, since VegT is able to rescue the effects of Cm-derriere, and both genes can transcriptionally activate each other in animal cap cells. Derriere appears to function in a loop with eFGF, VegT, and Xbra.

Development of Ventral and Posterior Patterning in the Endoderm

The signals that regulate the formation and patterning of the endoderm are less understood than those of the mesoderm and ectoderm. In amphibians, the commitment of cells to become endoderm appears to take place gradually from blastula to gastrula stages (Rodaway *et al.*, 1999; for reviews of endoderm development, see Wells and Melton, 1999; Alexander and Stainier, 1999; Grapin-Botton and Melton, 2000). The decision of mesendodermal cells to become mesoderm or endoderm might be regulated by factors secreted by the organizer in *Xenopus* or the node in higher vertebrates, although the nature of these factors remains largely unstudied (Wells and Melton, 1999).

In *Xenopus*, activin signaling appears important for the expression of several endodermal genes shown to have the ability to induce endodermal gene expression in embryonic cells, such as Mixer or the HMG-box transcription factors Sox17α and -β (see Wells and Melton, 1999; and references therein). Overexpression of Sox17 in the ectoderm leads to an induction of endoderm, whereas blocking Sox17 function leads to a conversion of endoderm into mesoderm, thus highlighting the notion that mesendodermal fates are regulated differentially by similar signaling events. Similarly, the paired-domain gene Mix-1 is a repressor of mesoderm and an inducer of endoderm in *Xenopus* (Hudson *et al.*, 1997; Lemaire *et al.*, 1998). Mix.1 is expressed in vegetal cells, and appears to form a negative regulatory loop with Xbra, although they are initially coexpressed in mesendodermal cells prior to gastrulation (Lemaire *et al.*, 1998). Unidentified factors must cooperate with Mix.1 in the development of the posterior, ventral endoderm, since expression of a transcriptional repressor EnR-Mix.1 fusion protein yields posterior endoderm defects (Lemaire *et al.*, 1998). There is also very strong evidence in *Xenopus* and zebrafish for a role of nodal-related members in endoderm formation (in *Xenopus*, see Yasuo and Lemaire, 1999; Clements *et al.*, 1999; in zebrafish, see Schier *et al.*, 1999; Feldman *et al.*, 1998; Alexander *et al.*, 1999), and the genetic analysis in zebrafish have clearly established an essential role of Nodal signaling for the formation of endodermal precursors.

Whereas the genetic analysis in zebrafish has also contributed extensively to our knowledge of the molecules required for initial endoderm formation downstream of nodal signaling, such as in the mutant fish *casanova* (unknown gene; Alexander *et al.*, 1999), *faust* (gata-5; Kikuchi *et al.*, 2000), and *bonnie and clyde* (Mix-like homeodomain containing protein; Reiter *et al.*, 1999), there is little information about the nature of the players involved in the establishment of molecular asymmetries along the D/V and A/P axis in the endoderm.

As described above, TGFβ signaling may be involved in the specification of all three germ layers (Henry *et al.*, 1996). Whereas high concentrations would lead to endoderm specification, lower doses would induce mesoderm formation, and the absence of activin/TGFβ signaling would promote ectodermal fates, as previously suggested (Hemmati-Brivanlou and Melton, 1992). The regionalization of the endoderm, however, appears to take place largely independently of mesoderm formation, and it may be prepatterned during the blastula stages. *Xenopus* explants obtained from UV-irradiated embryos have shown that the early development of the endoderm is also subject to a regionalization that takes place during cortical rotation, but these studies have been centered around dorsal endoderm markers (Henry *et al.*, 1996). These results suggested that anterior specification of the endoderm is linked to the establishment of the dorsal mesoderm through cortical rotation. In the mouse, the endoderm is clearly molecularly asymmetric along its anterior–posterior axis at the end of gastrulation, and the posterior endoderm expresses the intestinal fatty-acid-binding protein (IFAP), and *cdx-2*, a caudal-gene homolog (Wells and Melton, 1999), although in

general there is a lack of endodermal markers and the role of these genes in early endoderm patterning is unclear. Later patterning of the endoderm along the A/P and D/V axes appears to depend on the interactions of the endoderm with adjacent mesoderm and ectoderm regions (see Wells and Melton, 2000). Whereas anterior endodermal patterning is better understood, and the role of the anterior endoderm in patterning the anterior ectoderm has been well characterized (see Wells and Melton, 1999; and references therein for anterior endoderm development), knowledge about patterning of the posterior endoderm has lagged behind.

The role of FGF signaling in early endoderm patterning is unclear, and it may function, as in the mesoderm, to maintain the expression of endoderm-specific genes. Endoderm formation appears to be nonresponsive to FGF signaling in this model, both in *Xenopus* and zebrafish (Rodaway *et al.,* 1999; Jones *et al.,* 1996a). However, FGFs might also act to posteriorize the endoderm, as in the ectoderm, since FGF-4 induces posterior endoderm markers in a concentration-dependent manner in the mouse (Wells and Melton, 2000). Since expression of FGF-4 is found in the mouse primitive streak, it is likely that patterning of the endoderm, at least by midgastrulation, is affected by interactions between the endoderm and adjacent, regionalized mesoderm and ectoderm. However, the role of FGF signaling in endoderm patterning awaits the genetic ablation of FGF signaling in the endoderm.

Later embryological events, such as liver formation within the ventral endoderm, have been shown to depend on signals emanating from the adjacent cardiac mesoderm in both chicken and mouse (Le Douarin, 1968; Gualdi *et al.,* 1996). These signals appear to belong to the FGF family (Jung *et al.,* 1999). Similarly, pancreas development in chick appears to require signals from the notochord (Kim *et al.,* 1997). We know very little about the molecules that regulate the early posteriorization and ventralization of the endoderm, partially due to the lack of markers for posterior/ventral endoderm. This is an area that awaits further experimentation, although given the pace of the research in this field, we will not have to wait long for the answers.

CONCLUSION

The field of molecular embryology has seen a great advancement in our understanding of the signaling pathways that mediate germ-layer specification and patterning of the embryonic axis. This review has attempted to address our knowledge of the molecular mechanisms regulating posterior and ventral fate determination in the vertebrate embryo. Current data suggest that patterning of the three germ layers is coordinately regulated. Maternal and zygotic components in the early embryo influence the asymmetric distribution of functional protein domains, which in turn lead to the formation of signaling centers that then act to pattern all three germ layers. Although this has been extensively shown for the organizer/node in vertebrates, it is likely that other regions in the early embryo play critical roles (i.e., organizing) as well in patterning the ventral/posterior region.Whereas classical experimental embryology has taught us much about the potential for some tissues to act as "inducers" of particular tissue fates, it is ultimately molecular embryology which has provided mechanistic and conceptual insights into how such "inductive" processes take place in the embryo. Whereas we have traditionally thought of "inductive" processes as being driven by instructive signals, it is now clear that, at least for the biology of the organizer region, inhibitory signals play a dominant role in patterning all three germ layers. Provocatively, one can argue that similar mechanisms, or permissive signals, might operate in the remaining 300° of the pregastrula embryo to pattern the posterior and ventral regions. An interesting experiment carried out by Yamada (1950) showed that dissociated newt ventral marginal zone cells give rise to notochord and muscle (dorsal mesoderm), so maybe a new "default model," analogous to the one suggested for the nervous system, operates for the ventroposterior region of the embryo.

It is apparent that gradients of Activin/Nodal and BMP activities are critical for the earliest events in regionalizing all three germ layers. However, understanding how each of these gradients is established and maintained is far from being completed, and what the output from the functional interaction of these gradients is remains a challenge. Genetic mutation screens in zebrafish have complemented work in *Xenopus* and shown that components of these pathways are central to patterning the ventral and posterior region of the embryo. This review has highlighted that similar signaling molecules act in different germ layers to instruct dorsal versus ventral fates, and that the decision to become one cell type versus another depends on opposing decisions modulated through the transcriptional activation of different target transcription factors (for instance, Brachyury versus Mixer expression in the mesendoderm, as a simplified example of one of these decisions). The challenge now remains in placing this information in the global context of cell movements and windows of competence to respond to particular signals during development. The review presented here also highlights that the development of ventroposterior structures depends as much on instructive cues as on inhibitory signals and on the correct migratory movements of the cells during gastrulation (as evidenced in the studies with Brachyury and VegT), which, together with intrinsic signals that direct cell fate decisions, modulate the final outcome of these cells. A full understanding of how such complex processes take place can only come through a more comprehensive analysis of gene maps and morphogenetic movements at the time when the criticial decisions are occurring. Finally, it is the coordinate signaling interaction among all three germ layers which will finally determine the proper development of ventroposterior fates in the vertebrate embryo.

Although there has been great progress in recent years in the field of embryonic patterning, many issues remain to be

addressed. Teleologically, one must ask the seemingly simplest questions first. Why does the embryo devote such a large field (300° of its radial axis) to ventrolateral fates? Why does the anterior part form first, i.e., what regulates the timing of these events? Why are the posterior and ventral axis linked in vertebrates? From a molecular perspective, many questions arise from the gene expression patterns. Why are the inhibitors in the organizer? How many subdomains of gene expression exist in the ventral marginal zone? Is there an organizing center in the ventral side of the embryo? Do all cells within a subdomain of the VMZ acquire ventral or posterior fates? How much do morphogenetic movements contribute to the final fate acquisition of these cells? There is a need to correlate the available gene maps with fate maps and analyze the subdivisions of the marginal zone in terms of positional information. What is the default state of each one of these "compartments" of gene expression? How are these domains set up? Do they require maternal components? Is the asymmetry of gene expression patterns in the VMZ established prior to organizer formation, and if so, how do these genes affect the formation of the organizer in the DMZ?

More complex genetic analysis in the mouse and zebrafish will allow investigators to address the issue of redundancy of the players involved, as they are being identified. The models presented above for axis determination are based mostly on gain-of-function experiments, that take into account gene expression at the level of transcriptional regulation. We have little information about protein dynamics in the context of developmental events, at the level of threshold signaling and gradient formation. The issue of competence, or the window of time in which a tissue is able to respond to a particular inductive signal, is of particular importance. The question of post-transcriptional and post-translational regulation at the level of RNA stability, translational efficiency, and protein trafficking and degradation will become central to our understanding of how competence and responsiveness to particular signals in time and space will yield to patterning information and embryonic cell fate determination. It is certainly an exciting time for molecular embryologists.

ACKNOWLEDGMENTS

We thank Paul Wilson and Richard Harland for assistance with the fate map, Curtis Altmann for help with the figures, and Paul Wilson, Irene Zohn, and members of the Brivanlou lab for helpful suggestions on the manuscript.

REFERENCES

Agius, E., Oelgeschlager, M., Wessely, O., Kemp, C., and DeRobertis, E. M. (2000). Endodermal nodal-related signals and mesoderm induction in *Xenopus*. *Development* **127**, 1173–1183.

Alexander, J., and Stainier, D. Y. R. (1999). A molecular pathway leading to endoderm formation in zebrafish. *Curr. Biol.* **9,** 1147–1157.

Amacher, S. L., and Kimmel, C. B. (1998). Promoting notochord fate and repressing muscle development in zebrafish axial mesoderm. *Development* **125,** 1397–1406.

Amaya, E., Musci, T. J., and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryos. *Cell* **66,** 257–270.

Barth, K. A., Kishimoto, Y., Rohr, K. B., Seydler, C., Schulte-Merker, S., and Wilson, S. W. (1999). Bmp activity establishes a gradient of positional information throughout the entire neural plate. *Development* **126,** 4977–4987.

Bauer, H., Lele, Z., Rauch, G. J., Geisler, R., and Hammerschmidt, M. (2001) The type I. serine/threonine kinase receptor Alk8/Lost-a-fin is required for Bmp2b/7 signal transduction during dorsoventral patterning of the zebrafish embryo. *Development* **128,** 849–858.

Beddington, R. S. P., and Robertson, E. J. (1999). Axis development and early asymmetry in mammals. *Cell* **96,** 195–209.

Bolce, M. E., Hemmati-Brivanlou, A., Kushner, P. D., and Harland, R. M. (1992). Ventral ectoderm of *Xenopus* forms neural tissue, including hindbrain, in response to activin. *Development* **115,** 681–688.

Casey, E. S., Tada, M., Fairclough, L., Wylie, C., Heasman, J., and Smith, J. C. (1999). Bix4 is activated directly by VegT and mediates endoderm formation in *Xenopus* development. *Development* **126**, 4193–4200.

Chalmers, A. D., and Slack, J. M. W. (2000) The *Xenopus* tadpole gut: Fate maps and morphogenetic movements. *Development* **127,** 381–392.

Christian J. L., and Moon R. T. (1993). Interactions between Xwnt-8 and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. *Genes Dev.* **7,** 13–28.

Clements, D., Friday, R. V., and Woodland, H. R. (1999). Mode of action of VegT in mesoderm and endoderm formation. *Development* **126,** 4903–4911.

Crease, D. J., Dyson, S., and Gurdon, J. B. (1998). Cooperation between the activin and Wnt pathways in the spatial control of organizer gene expression. *Proc. Natl. Acad. Sci. USA* **95,** 4398–4403.

Conlon, F. L., Lyons, K. M., Takaesu, N., Barth, K. S., Kispert, A., Hermann, B., and Robertson, E. J. (1994). A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. *Development* **120,** 1919–1928.

Conlon, F. L., Sedgwick, S. G., Weston, K. M., and Smith, J. C. (1996). Inhibition of Xbra transcription activation causes defects in mesodermal patterning and reveals autoregulation of Xbra in dorsal mesoderm. *Development* **122,** 2427–2435.

Conlon, F. L., and Smith, J. C. (1999). Interference with brachyury function inhibits convergent extension, causes apoptosis, and reveals separate requirements in the FGF and activin signalling pathways. *Dev. Biol.* **213,** 85–100.

Dale, L., and Slack, J. M. W. (1987a). Fate map for the 32-cell stage of *Xenopus laevis*. *Development* **99,** 527–551.

Dale, L., and Slack, J. M. W. (1987b). Regional specification within the mesoderm of early embryos of *Xenopus laevis*. *Development* **100,** 279–295.

Dale, L., Howes, G., Price, B. M. J., and Smith, J. C. (1992). Bone morphogenetic protein-4: A ventralizing factor in early *Xenopus* development. *Development* **115,** 573–585.

Deblandre, G. A., Wettstein, D. A., Koyano-Nakagawa, N., and Kintner, C. (1999). A two-step mechanism generates the spacing

pattern of the ciliated cells in the skin of Xenopus embryos. *Development* **126,** 4715–4728.

Dick., A., Meier, A., and Hammerschmidt, M. (1999). Smad1 and Smad5 have distinct roles during dorsoventral patterning of the zebrafish embryo. *Dev. Dyn.* **216,** 285–298.

Dick, A., Hild, M., Bauer, H., Imai, Y., Maifeld, H., Schier, A. F., Talbot W. S., Bouwmeester, T., and Hammerschmidt, M. (2000). Essential role of Bmp7 (snailhouse) and its prodomain in dorsoventral patterning of the zebrafish embryo. *Development* **127,** 343–354.

Ding, J., Yang, L., Yan, Y-T., Chen, A., Desai, N., Wynshaw-Boris, A., and Shen, M. M. (1998). Cripto is required for correct orientation of the anterior-posterior axis in the mouse embryo. *Nature* **395,** 702–707.

Dong, Z., Xu, R., Kim, J., Zhan, S., Ma, W., Colburn, N. H., and Kung, H/. (1996). AP-1/Jun is required for early *Xenopus* development and mediates mesoderm induction by fibroblast growth factor but not by activin. *J. Biol. Chem.* **271,** 9942–9946.

Feldman, B., Gates, M. A., Egan, E. S., Dougan, S. T., Rennebeck, G., Sirotkin, H. I., Schier, A. F., and Talbot, W. S. (1998). Zebrafish organizer development and germ-layer formation require nodal-related signals. *Nature* **395,** 181–185.

Fredieu, J. R., Cui, Y. Maier, D., Danilchik, M. V., and Christian, J. L. (1997). Xwnt-8 and lithium act upon either dorsal mesodermal or neurectodermal cells to cause a loss of forebrain in *Xenopus* embryos. *Dev. Biol.* **186**, 100–114.

Gammill, L. S., and Sive, H. (2000). Coincidence of otx2 and BMP4 signaling correlates with Xenopus cement gland formation. *Mech. Dev.* **92,** 217–226.

Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rowning, B., and Stewart, R. (1989). Cortical rotation of the *Xenopus* egg: Consequences for the anteriorposterior pattern of embryonic dorsal development. *Development* **107**(Suppl.), 37–51.

Graff, J. M., Bansal, A., and Melton, D.A. (1996). *Xenopus* Mad proteins transduce distinct subsets of signals for the TGF beta superfamily. *Cell* **85,** 479–487.

Grapin-Botton, and Melton, D. A. (2000). Endoderm development, from patterning to organogenesis. *Trends Genet.* **16,** 124–130.

Green, J. B., and Smith, J. C. (1990). Graded changes in dose of a Xenopus activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature* **347,** 391–394.

Griffin, K., Patient, R., and Holder, N. (1995). Analysis of FGF function in normal and no tail zebrafish embryos reveals separate mechanisms for formation of the trunk and the tail. *Development* **121,** 2983–2994.

Griffin, K J. P., Amacher, S. L., Kimmel, C. B., and Kimelman, D. (1998). Molecular identification of spadetail: Regulation of zebrafish trunk and tail mesoderm formation by T-box genes. *Development.* **125,** 3379–3388.

Gritsman, K., Zhang, J., Cheng, S., Heckscher, E., Talbot, W. S., and Schier, A. F. (1999). The EGF-CFC protein one-eye-pinhead is essential for nodal signaling. *Cell* **97,** 121–132.

Gritsman, K., Talbot, W. S., and Schier, A. T. (2000). Nodal signaling patterns the organizer. *Development* **127,** 921–932.

Gualdi, R., Bossard. P., Zheng, M., Hamada, Y., Coleman, J. R., and Zaret, K. S. (1996). Hepatic specification of the gut endoderm in vitro: Cell signaling and transcriptional control. *Genes Dev.* **10,** 1670–1682.

Halpern, M. E., Ho, R. K., Walker, C., and Kimmel, C. B. (1993). Induction of muscle pioneers and floor plate is distinguished by the xebrafish no tail mutation. *Cell* **75,** 99–111.

Hansen, C. S., Marion, C. D., Steele, K., George, S., and Smith, W. C. (1997). Direct neural induction and selective inhibition of mesoderm and epidermis inducers by Xnr3. *Development.* **124,** 483–492.

Harland, R., and Gerhart, J. (1997). Formation and function of Spemann's organizer. *Annu. Rev. Cell Dev. Biol.* **13,** 611–667.

Hatada, Y., and Stern, C. (1994). A fate map of the epiblast of the early chick embryo. *Development* **120,** 2879–2889.

Heisenberg, C-P., Tada, M., Rauch, G-J., Saude, L., Concha, M. L., Geisler, R., Stemple, D. L., Smith, J. C., and Wilson, S. W. (2000) Silberblick/Wnt11. mediates convergent extension movements during zebrafish gastrulation. *Nature* **405,** 76–81.

Helde, K. A., Wilson, E. T., Cretekos, C. J., and Grunwald, D. J. (1994). Contribution of early cells to the fate map of the zebrafish gastrula. *Science* **265,** 517–520.

Hemmati-Brivanlou, A., and Melton, D. A. (1992). A truncated activin receptor inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos. *Nature* **359**, 609–614.

Henry, G. L., Brivanlou, I. H., Kessler, D. S., Hemmati-Brivanlou, A., and Melton, D. (1996). TGF-β signals and a prepattern in *Xenopus laevis* endodermal specification. *Development* **122**, 1007–1015.

Herrman, B. G., Labeit, S., Poutska, A., King, T. R., and Lehrach, H. (1990). Cloning of the T gene is required for mesoderm formation in the mouse. *Nature* **343,** 617–622.

Hild, M., Dick, A., Rauch, G-J., Meier, A., Bouwmeester, T., Haffter, P., and Hammerschmidt, M. (1999). The smad5 mutation somitabun blocks bmp2b signaling during early dorsoventral patterning of the zebrafish embryo. *Development* **126,** 2149–2159.

Hudson, C., Clemens, D., Friday, R. V., Stott, D., and Woodland, H. R. (1997). Xsox17-alpha and -beta mediate endoderm formation in *Xenopus. Cell* **91**, 397–405.

Isaacs, H. V., Pownhall, M. E., and Slack, J. M. W. (1994). eFGF regulates Xbra expression during *Xenopu*s gastrulation. *EMBO J.* **13,** 4469–4481.

Jones, C. M., Lyons, K. M., Lapan, P. M., Wright, C. V. E., and Hogan, B. L. M. (1992). DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizingf factor in *Xenopus* mesoderm induction. *Development* **115**, 639–647.

Jones, C. M., Armes, N., and Smith, J. C. (1996a). Signaling by TGF-b family members: Short-range effects of Xnr-2 and BMP-4 contrast with the long-range effects of activin. *Curr. Biol.* **6,** 1468–1475.

Jones, C. M., Dale, L., Hogan, B. L., Wright, C. V., and Smith, J. C.(1996b). Bone morphogenetic protein-4 (BMP-4) acts during gastrula stages to cause ventralization of Xenopus embryos. *Development.* **122,** 1545–1554.

Jung, J., Zheng, M., Goldfarg, M., and Zaret, K. S. (1999). Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* **284,** 1998–2003.

Kao, K. R., and Elinson, R. P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* **127,** 64–77.

Keller, R. (1975). Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. I. Prospective areas and morphogenetic movements of the superficial layer. *Dev. Biol.* **42,** 222–241.

Keller, R. (1976). Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. II. Prospective areas and morphogenetic movements of the deep layer. *Dev. Biol.* **51,** 118–137.

Keller, R. (1991). Early embryonic development of *Xenopus laevis. Methods Cell Biol.* **36,** 61–113.

Kessler, D. S., and Melton, D. A. (1994). Vertebrate embryonic induction: Mesodermal and neural patterning. *Science* **266,** 596–604.

Kiecker, C., Muller, F., Wu, W., Glinka, A., Strahle, U., and Niehrs, C. (2000). Pheotypic effects in *Xenopus* and zebrafish suggest that *one-eye-pinhead* functions as antagonist of BMP signaling. *Mech. Dev.* **94**, 37–46.

Kim, S. K., Hebrok, M., and Melton, D. A. (1997). Notochord to endoderm signaling is required for endoderm development. *Development* **124,** 4234–4252.

Kimmel, C. B., Kane, D. A., Walker, C., Warga, R. M., and Rothman, M. B. (1989). A mutation that changes cell movement and cell fate in the zebrafish embryo. *Nature* **337,** 358–362.

Kimmel, C. B., Warga, R. M., and Schilling, T. F. (1990). Origin and organization of the zebrafish fate map. *Development* **108,** 581–594.

Kikuchi, Y., Trinh, L. A., Reiter, J. F., Alexander, J., Yelon, D., and Stainier, D. Y. R. (2000). The zebrafish bonnie and clyde gene encodes a Mix family homeodomain protein that regulates the generation of endodermal precursors. *Genes Dev.* **14,** 1279–1289.

Kishimoto, Y., Lee, K. H., Zon, L., Hammerschmidt, M., and Schulte-Merker, S. (1997). The molecular nature of zebrafish swirl: BMP2 function is essential during early dorsoventral patterning. *Development* **124,** 4457–4466.

Klein, P. S., and Melton, D. A. (1996). A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. USA* **93,** 8455–8459.

Kolm, P. J., and Sive, H. L. (1995). Regulation of the *Xenopus* labial homeodomain genes, HoxA1 and HoxD1: Activation by retinoids and peptide growth factors. *Dev. Biol.* **167,** 34–49.

Kuhl, M., Shedahl, L. C., Malbon, C. C., and Moon, R. T. (2000) Ca/calmodulin-dependent protein kinase II is stimulated by Wnt and frizzled homologs and promotes ventral cell fates in *Xenopus. J. Biol. Chem.* **275**, 12701–12711.

Kumano, G., Belluzzi, L., and Smith, W. C. (1999). Spatial and temporal properties of ventral blood island induction in *Xenopus laevis. Development* **126**, 5327–5337.

Lane, M. C., and Smith, W. C. (1999). The origins of the primitive blood in *Xenopus*: Implications for axial patterning. *Development* **126**, 423–434.

Larabell, C. A., Torres, M., Rowning, B. A., Yost, C., Miller, J. R., Wu, M., Kimelman, D., and Moon, R. T. (1997). Establishment of the dorso-ventral axis in Xenopus embryos is presaged by early asymmetries in beta-catenin that are modulated by the Wnt signaling pathway. *J. Cell Biol.* **136,** 1123–1136.

Laurent, M. N., and Cho, K. W. Y. (1999). Bone morphogenetic protein antagonism of Spemann's organizer is independent of Wnt signaling. *Dev. Biol.* **206,** 157–162.

Lawson, K. A., Meneses, J. J., and Pedersen, R. A. (1991). Clonal analysis of epiblast fate during germ-layer formation in the mouse embryo. *Development* **113,** 891–991.

Le Douarin, N. (1968). Synthesis of glycogen in hepatocytes undergoing differentiation: Role of homologous and heterologous mesenchyma. *Dev. Biol.* **17**, 101–114.

Lemaire, P., Darras, S., Caillo, D., and Kodjabachian, L. (1998). A role for the vegetally expressed *Xenopus* gene Mix.1 in endoderm formation and in the restriction of mesoderm to the marginal zone. *Development* **125,** 2371–2380.

Liu, P., Wakamiya, M., Shea, M. J., Albretch, U., Behringer, R. R., and Bradley, A. (1999). Requirement for Wnt3a in vertebrate axis formation. *Nat. Genet.* **22,** 361–365.

Malacinski, G. M., Brothers, A. J., and Chung, H. M. (1975). Desruction of components of the neural induction system of the amphibian egg with ultraviolet irradiation. *Dev. Biol.* **56,** 24–39.

Massague, J., and Chen, Y. (2000) Controling TGF-β signaling. *Genes Dev.* **14,** 627–644.

Matzuk, M. M., Kumar, T. R., Vassalli, A., Bickenbach, J. R., Roop, D. R., Jaenisch, R., and Bradley, A. (1995). Functional analysis of activins during mammalian development. *Nature* **374,** 354–356.

McDowell, N., Zorn, A. M., Crease, D. J., and Gurdon, J. B. (1997). Activin has direct long-range signaling activity and can form a concentration gradient by diffusion. *Curr. Biol.* **7,** 671–681.

Mintzer, K., Lee, M. A., Runke, G., Trout, J., Whitman, M., and Mullins, M. C. (2001). Lost-a-fin encodes a type I BMP receptor, Alk8, acting maternally and zygotically in dorsoventral pattern formation. *Development* **128,** 859–869.

Moody, S. A. (1987). Fates of the blastomeres of the 32-cell-stage *Xenopus* embryo. *Dev Biol.* **122,** 300–319.

Moon, R. T., Brown, J. D., and Torres, M. (1997). Wnts modulate cell fate and behavior during vertebrate development. *Trends Genet.* **13,** 157–162.

Mullins, M. C., Hammerschmidt, M., Kane, D. A., Odenthal, J., Brand, M., van Eeden, F. J., Furutani-Seiki, M., Granato, M., Haffter, P., Heisenberg, C. P., Jiang, Y. J., Kelsh, R. N., and Nusslein-Volhard, C. (1996). Genes establishing dorsoventral pattern formation in the zebrafish embryo: The ventral specifying genes. *Development* **123,** 81–93.

Neave, B., Holder, N., and Patient, R. (1997). A graded response to BMP-4 spatially coordinates patterning of the mesoderm and ectoderm in the zebrafish. *Mech. Dev.* **62,** 183–195.

Nguyen, V. H., Schmid, B., Trout, J., Connors, S. A., Ekker, M., and Mullins, M. C. (1998). Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a bmp2/swirl pathway of genes. *Dev. Biol.* **199,** 93–110.

Nieuwkoop, P. D. (1969a). The formation of the mesoderm in Urodelean amphibians. I. Induction by endoderm. *Roux's Arch. Entw. Mech. Org.* **162,** 298–315.

Nieuwkoop, P. D. (1969b). The formation of the mesoderm in urodelean amphibians. II. The origin of dorso-ventral polarity of the mesoderm. *Roux's Arch.* **163,** 298–315.

Nikaido, M., Tada, M., Takeda, H., Kuroiwa, A., and Ueno, N. (1999). In vivo analysis using variants of zebrafish BMPR-IA: Range of action and involvement of BMP in ectodermal patterning. *Development* **126**, 181–190.

Peifer, M., and Polakis, P. (2000). Wnt signaling in oncogenesis and embryogenesis: A look outside the nucleus. *Science* **287,** 1606–1609.

Read, E. M., Rodaway, A. R., Neave, B., Brandon, N., Holder, N., Patient, R. K., and Walmsley, M. E. (1998). Evidence for non-axial A/P patterning in the nonneural ectoderm of Xenopus and zebrafish pregastrula embryos. *Int. J. Dev. Biol.* **42,** 763–774.

Reilly, K. M., and Melton, D. A. (1996). Short-range signaling by candidate morphogens of the TGFβ family and evidence for a relay mechanism of induction. *Cell* **86,** 743–754.

Reiter, J. F., Alexander, J., Rodaway, A., Yelon, D., Patient, R., Holder, N., and Stainier, D. Y. R. (1999). Gata5 is rquired for the development of the heart and endoderm in zebrafish. *Genes Dev.* **13,** 2983–2995.

Rodaway, A., and Patient, R. (2001) Mesendoderm: An ancient germ layer? *Cell* **105,** 169–172.

Rodaway, A., Takeda, H., Koshida, S., Broadbent, J., Price, B., Smith, J. C., Patient, R., and Holder, N. (1999). Induction of the mesendoderm in the zebrafish germ ring by yolk cell-derived

TGF-β family signals and discrimination of mesoderm and endoderm by FGF. *Development* **126,** 3067–3078.

Ryan, K., Garrett, N., Mitchell, A., and Gurdon, J. B. (1996). Eomesodermin, a key early gene in Xenopus mesoderm differentiation. *Cell* **87,** 989–1000.

Ryan, K., Garrett, N., Bourillot, P., Stennard, F., and Gurdon, J. B. (2000). The *Xenopus Eomesodermin* promoter and its concentration-dependent response to activin. *Mech. Dev.* **94,** 133–146.

Russ, A. P., Wattler, S., Colledge, W. H., Aparicio, S. A., Carlton, M. B., Pearce, J. J., Barton, S. C., Surani, M. A., Ryan, K., Nehls, M. C., Wilson, V., and Evans, M. J. (2000) *Eomesodermin* is required for mouse trophoblast development and mesoderm formation. *Nature* **404,** 95–99.

Salic, A. N., Kroll, K. L., Evans, L. M., and Kirschner, M. W. (1997). *Sizzled*: A secreted Xwnt8 antagonist expressed in the ventral marginal zone of *Xenopus* embryos. *Development* **124,** 4739–4748.

Schier, A. F., Neuhauss, S. C., Helde, K. A., and Talbot, W. S. (1997). The one-eye pinhead gene functions in mesoderm and endoderm formation in zebrafish and interacts with no tail. *Development* **124,** 327–342.

Schier, A. F., and Shen, M. M. (2000). Nodal signalling in animal development. *Nature* **403,** 385–389.

Schlosser G., and Northcutt, R. G. (2000). Development of neurogenic placodes in *Xenopus laevis*. *J. Comp. Neurol.* **418,** 121–146.

Schmid, B., Furthauer, M., Connors, S. A., Trout, J., Thisse, B., Thisse, C., and Mullins, M. C. (2000). Equivalent genetic roles for bmp7/snailhouse and bmp2b/swirl in dorsoventral pattern formation. *Development* **127,** 957–967.

Schulte-Merker, S., and Smith, J. C. (1995). Mesoderm formation in response to brachyury requires FGF signaling. *Curr. Biol.* **5,** 62–67.

Smith, J. (1989). Mesoderm induction and mesoderm-inducing factors in early amphibian development. *Development* **105,** 665–677.

Smith, J. L., Gesteland, K. M., and Schoenwolf, G. C. (1994). Prospective fate map of the mouse primitive streak at 7.5 days of gestation. *Dev. Dyn.* **201,** 279–289.

Song, J., Oh, S. P., Schrewe, H., Nomura, M., Lei, H., Okano, M., Gridley, T., and Li, E. (1999). The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. *Dev. Biol.* **213,** 157–169.

Stachel, S. E., Grunwald, D. J., and Myers, P. Z. (1993). Lithium perturbation and goosecoid expression identify a dorsal specification pathway in the pregastrula zebrafish. *Development* **117**, 1261–1274.

Strehlow, D., Heinrich, G., and Gilbert, W. (1994). The fates of the blastomeres of the 16-cell zebrafish embryo. *Development* **120,** 1791–1798.

Sun, B. I., Bush, S. M., Collins-Racie, L. A., LaVallie, E. R., DiBlasio-Smith, E. A., Wolfman, N. M., McCoy, J. M., and Sive, H. L. (1999). Derriere: A TGF-β family member required for posterior development in *Xenopus*. *Development* **126,** 1467–1482.

Suzuki, A., Chang, C., Yingling, J. M., Wang, X.-F., and Hemmati-Brivanlou, A. (1997a). Smad5 induces ventral fates in the *Xenopus* embryo. *Dev. Biol.* **184,** 402–405.

Suzuki, A., Ueno, N., and Hemmati-Brivanlou, A. (1997b). *Xenopus* msx1 mediates epidermal induction and neural inhibition by BMP4. *Development* **124,** 3037–3044.

Tada, M., and Smith, J. C. (2000) Xwnt11 is a target of *Xenopus* brachyury:regulation of gastrulation movements via dishevelled, but not through the canonical Wnt pathway. *Development* **127,** 2227–2238.

Tada, M., Casey, E. S., Fairclough, L., and Smith, J. C. (1998). Bix1, a direct target of *Xenopus* T-box genes, causes formation of ventral mesoderm and endoderm. *Development* **125,** 3997–4006.

Takahashi, S., Yokota, C., Takano, K., Tanegashima, K., Onuma, Y., Goto, J., and Asashima, M. (2000). Two novel nodal-related genes initiate early inductive events in Xenopus Nieuwkoop center. *Development* **127,** 5319–5329.

Thisse, B., Wright, C. V. E., and Thisse, C. (2000) Activin-. and nodal-related factors control anterior-posterior patterning of the zebrafish embryo. *Nature* **403,** 425–428.

Vodicka, M. A., and Gerhart, J. C. (1995). Blastomere derivation and domains of gene expression in the Spemann organizer of *Xenopus laevis*. *Development* **121,** 3505–3518.

von Bubnoff, A., Schmidt, J. E., and Kimelman, D. (1995). The *Xenopus laevis* homeobox gene Xgbx-2 is an early marker of anteroposterior patterning of the ectoderm. *Mech. Dev.* **54,** 149–160.

Wada, S., Katsuyama, Y., and Saiga, H. (1999). Anteroposterior patterning of the epidermis by inductive influences from the vegetal hemisphere cells in the ascidian embryo. *Development* **126,** 4955–4963.

Warga, R. M., and Nusslein-Volhard, C. (1999). Origin and development of the zebrafish endoderm. *Development* **126,** 827–838.

Weinstein, D. C., Honore, E., and Hemmati-Brivanlou, A. (1997). Epidermal induction and inhibition of neural fate by translation initiation factor 4AIII. *Development* **124,** 4235–4242.

Weinstein, D. C., Marden, J., Carnevalli, F., and Hemmati-Brivanlou, A. (1998). FGF-mediated mesoderm induction involves the Src-family kinase Laloo. *Nature* **394,** 904–908.

Weinstein, D. C., and Hemmati-Brivanlou, A. (1999). Neural induction. *Annu. Rev. Cell Dev. Biol.* **15,** 411–433.

Wells, J. M., and Melton, D. A. (1999). Vertebrate endoderm development. *Annu. Rev. Cell Dev. Biol.* **15,** 393–410.

Wells, J. M., and Melton, D. A. (2000). Early mouse endoderm is patterned by soluble factors from adjacent germ layers. *Development* **127,** 1563–1572.

Wetts, R., and Fraser, S. E. (1989). Slow intermixing of cells during *Xenopus* embryogenesis contributes to the consistency of the blastomere fate map. *Development* **105,** 9–15.

Wilson, V., Manson, L., Skarnes, W. C., and Beddington, R. S. P. (1995). The T gene is necessary for normal mesodermal morphogenetic cell movements during gastrulation. *Development* **121,** 877–886.

Wilson, P. A., and Hemmati-Brivanlou, A. (1997). Vertebrate neural induction: Inducers, inhibitors and a new synthesis. *Neuron* **18**, 699–710.

Winnier, G., Blessing, M., Labosky, P. A., and Hogan, B. L. M. (1995). Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev.* **9,** 2105–2116.

Winklbauer, R. (1989). Development of the lateral line system in Xenopus. *Prog. Neurobiol.* **32,** 181–206.

Yamada, T. (1950). Dorsalization of the ventral marginal zone of triturus gastrua. I. Ammonia treatment of the medio-ventral marginal zone. *Biol. Bull.* **98,** 98–121.

Yamada, K., Takabake, Y., Takabatake, T., and Takeshima, K. (1999). The early expression control of Xepsin by nonaxial and

planar posteriorizing signals in *Xenopus* epidermis. *Dev. Biol.* **214**, 318–330.

Yamaguchi, Y., and Shinagawa, A. (1989). Marked alteration at midblastula transition in the effect of lithium on formation of the larval body pattern of *Xenopus laevis*. *Dev. Growth Differ.* **31,** 531–541.

Yasuo, H., and Lemaire, P. (1999). A two-step model for the fate determination of presumptive endodermal blastomeres in ≈enopus embryos. *Curr. Biol.* **9,** 869–879.

Zhang, H., and Bradley, A. (1996). Mice deficient for BMP-2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* **122,** 2977–2986.

Zhang, J., Houston, D. W., King, M. L., Payne, C., Wylie, C., and Heasman, J. (1998). The role of maternal VegT in establishing the primary germ layers in *Xenopus* embryos. *Cell* **94**, 515–524.

Received for publication August 24, 2000
Revised June 18, 2001
Accepted June 18, 2001
Published online July 31, 2001